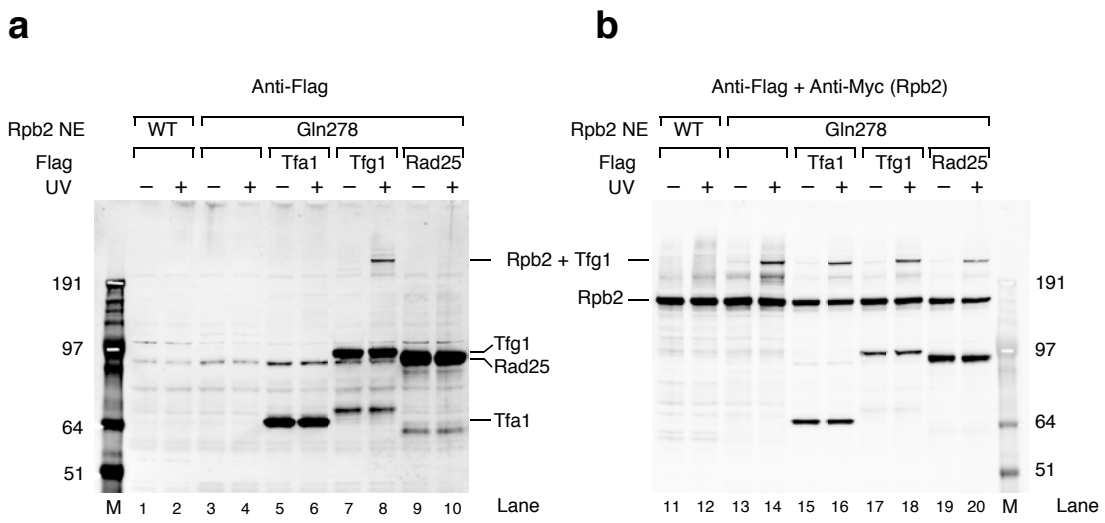
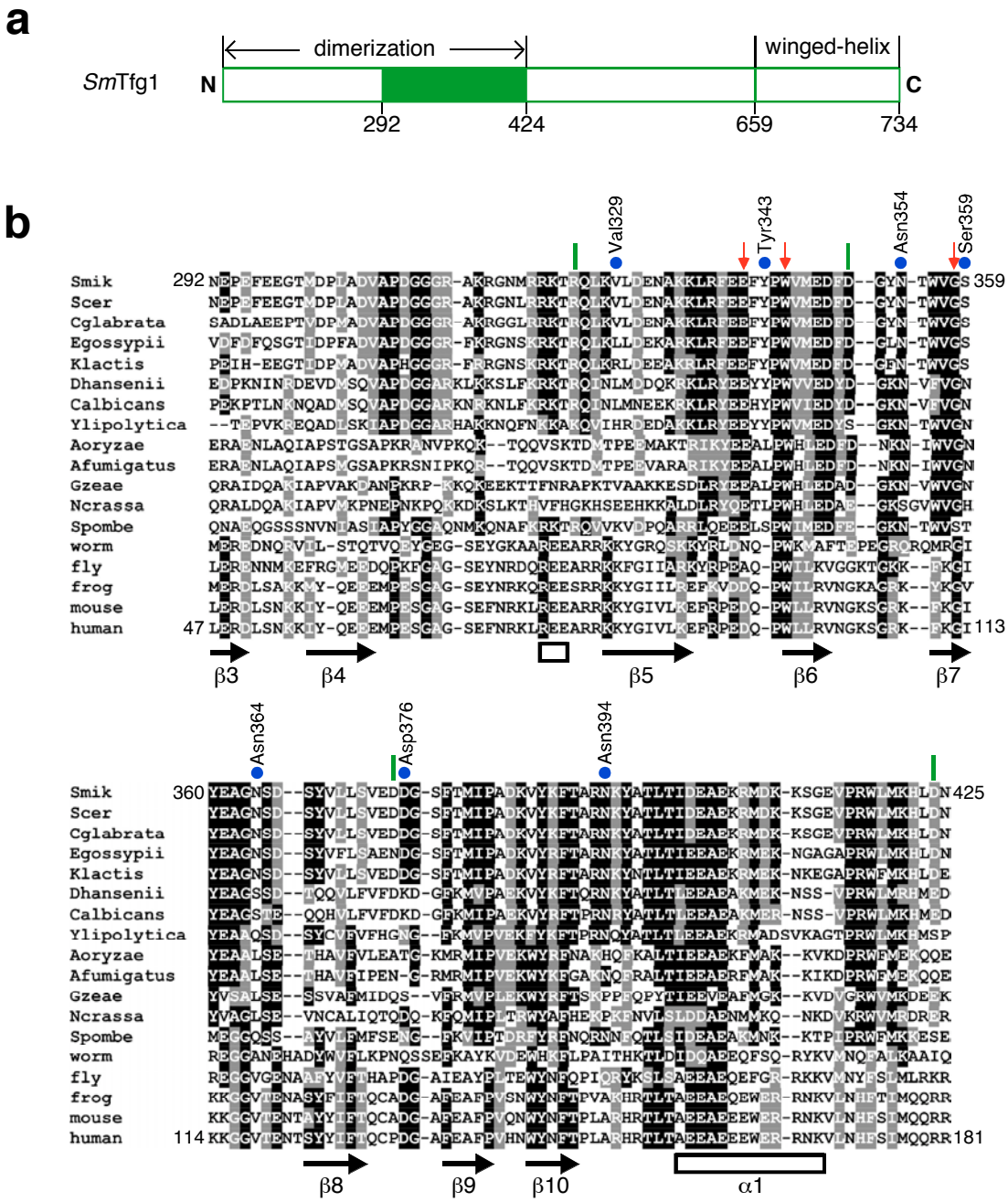
Supplementary Figure 1.



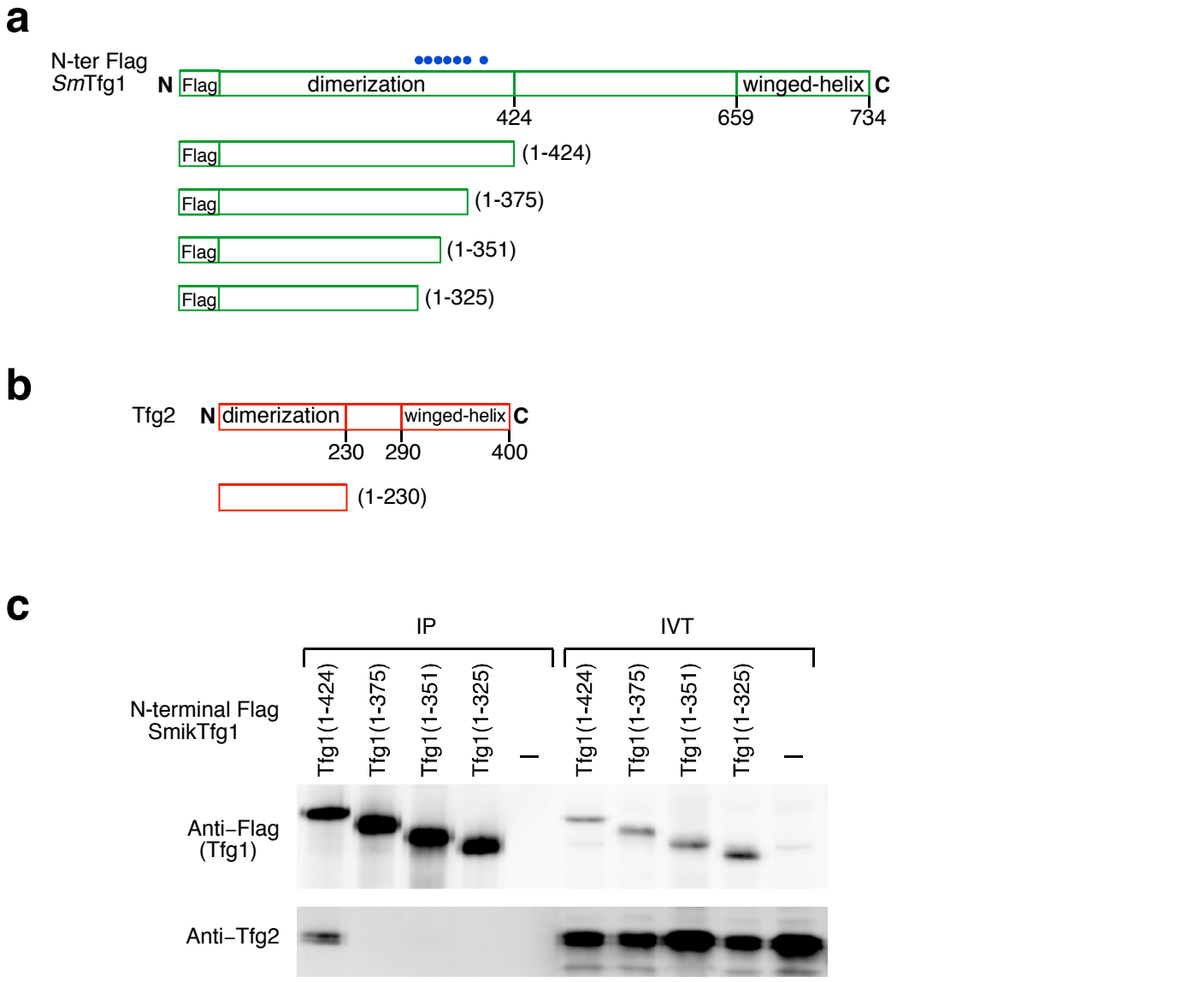
Supplementary figure 1. Identification of the protein crosslinked to Rpb2 Gln278-Bpa. (a) Nuclear extracts from the indicated Flag epitope tagged strain and the Rpb2 amber strain Gln278-Bpa were pre-mixed and used in the immobilized template assay for PIC formation and photocrosslinking. Protein bands recognized by the anti-Flag antibody are indicated. Crosslinking between Tfg1 and Rpb2-Gln278 is identified based on the slow mobility band (Rpb2+Tfg1) recognized by both anti-Flag antibody and anti-Myc antibody (Rpb2; see (b)). Rpb2 NE: Rpb2 nuclear extract from either wild-type (WT; no amber codon for Bpa incorporation) or Rpb2 Gln278-Bpa. Flag: C-terminal Flag epitope tagged protein. UV: UV irradiation. M: molecular weight marker. (b) The same Western blot was probed with anti-Myc antibody against Myc epitope tagged Rpb2.

Supplementary Figure 2.



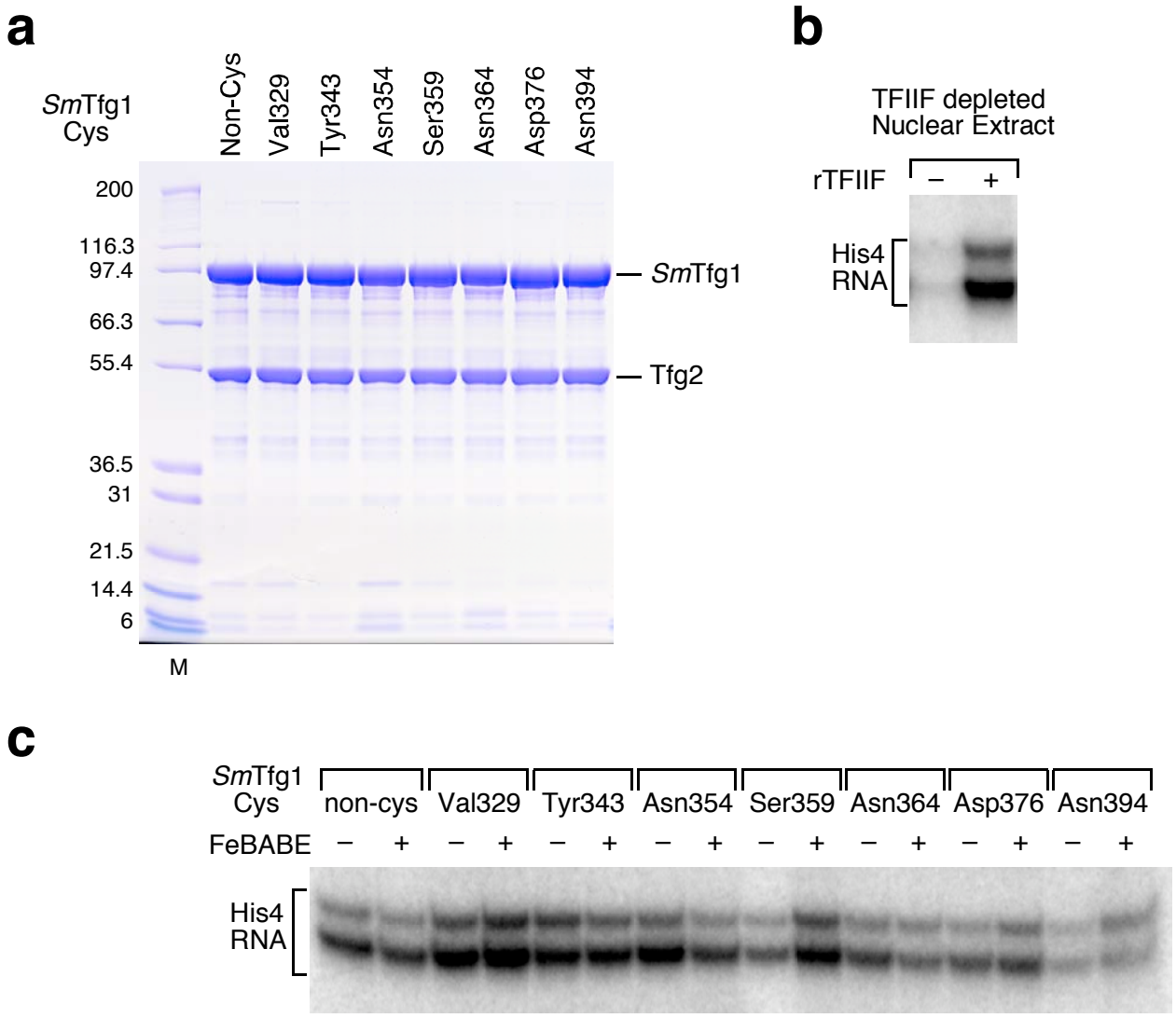
Supplementary figure 2. Comparison of Tfg1 dimerization domains. (a) Schematic representation of the Tfg1 subunit. The numbering refers to the residues in S. mikatae Tfg1 (SmTfg1). Amino acid sequence ranges of the dimerization and winged-helix domains are based on multiple sequence alignment (see below and data not shown). The highly conserved region within the dimerization domain is colored green. (b) Sequence alignment of the highly conserved region in Tfg1 (Rap74 in human) dimerization domain. The alignment was generated using the program Clustal X. The secondary structural elements of human Rap74 structure (PDB code: 1F3U) are listed below with arrows and bars indicating beta-strand and helix, respectively. Blue dots denote cysteine mutations in SmTfg1 for site-specific conjugation with the protein cleavage reagent FeBABE. Red vertical arrows indicate mutations that alter the preference for transcription start to upstream. Green vertical lines denote the last amino acids of the SmTfg1 N-terminal protein fragments for co-immune precipitation with the Tfg2 dimerization domain (see Supplementary Figure 3 below). The amino acid numbers for SmTfg1 and human Rap74 are indicated. The sequences are from organisms: Scer, Saccharomyces cerevisiae; Smik, Saccharomyces mikatae; Cglabrata, Candida glabrata; Egossypii, Eremothecium gossypii; Klactis, Kluyveromyces lactis; Dhansenii, Debaryomyces hansenii; Calbicans, Candida albicans; Ylipolytica, Yarrowia lipolytica; Aoryzae, Aspergillus oryzea; Afumigatus, Aspergillus fumigatus; Gzeae, Gibberella zeae; Ncrassa, Neurospora crassa; Spombe, Schizosaccharomyces pombe; human, Homo sapiens; mouse, Mus musculus; frog, Xenopus laevis; fly, Drosophila melanogaster; worm, Caenorhabditis elegans.

Supplementary Figure 3.



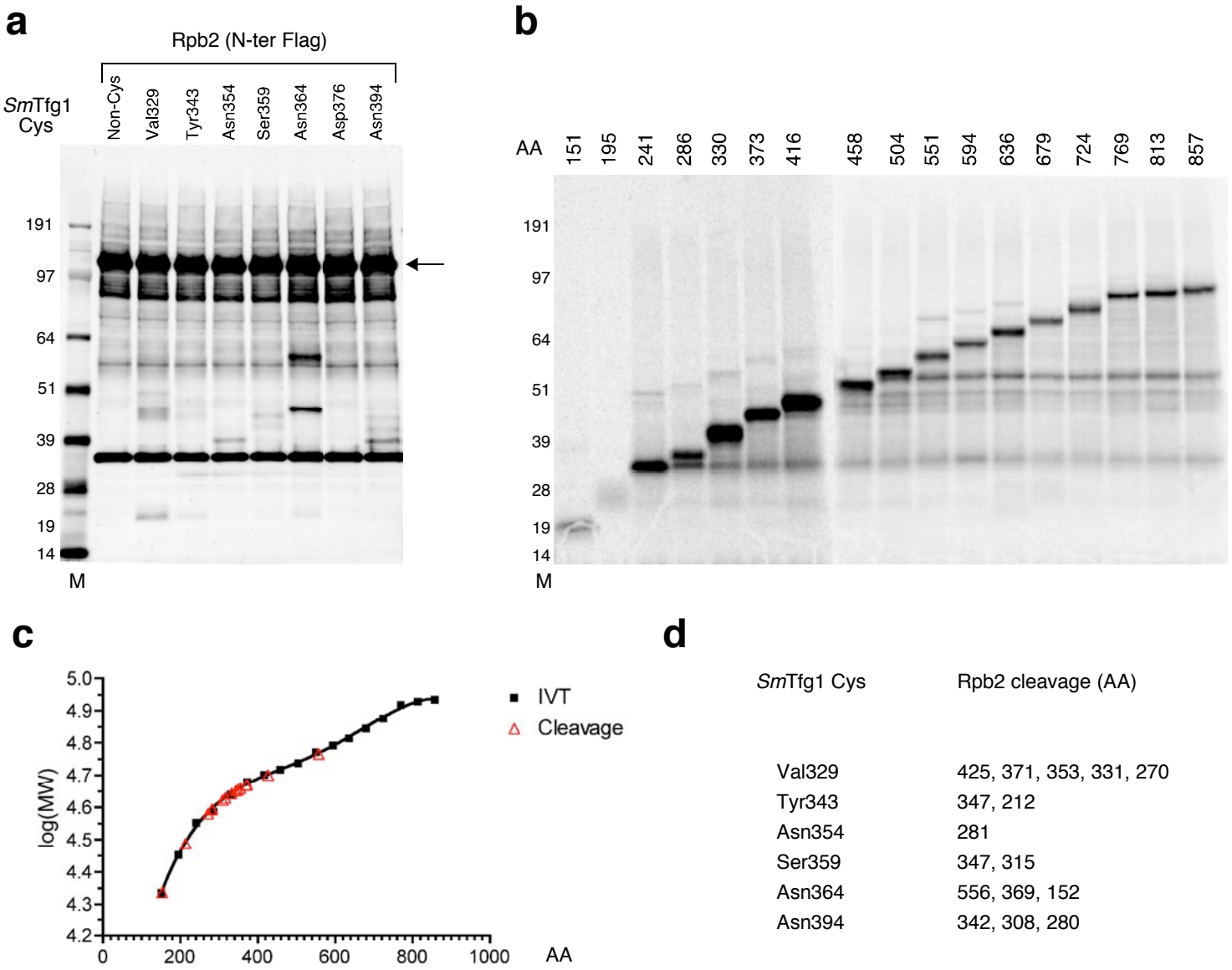
Supplementary figure 3. Localization of the Tfg1 and Tfg2 dimerization domains. (a) Schematic representation of the N-terminal Flag-tagged Tfg1. The numbering refers to SmTfg1 residues. The four peptide fragments generated by in vitro translation and used for co-immune precipitation with the Tfg2 dimerization domain are listed below. Blue dots denote the locations of cysteine mutations in SmTfg1 (see Supplementary Fig. 2b). (b) Schematic representation of S. cerevisiae Tfg2. The amino acid numbers of the dimerization and winged-helix domains are based on multiple sequence alignment (data not shown). The peptide fragment (aa 1-230) of ScTfg2 used for co-immune precipitation is indicated. (c) Co-expression and co-immune precipitation of SmTfg1 and ScTfg2 derivatives. The SmTfg1 and Tfg2 derivatives were co-expressed using in vitro translation (IVT) with PCR-generated DNAs encoding the indicated amino acid residues and visualized by Western analysis using anti-Flag and Tfg2 antibodies.
The results demonstrate that Tfg1 1-424 and Tfg2 1-230 dimerize as predicted by sequence alignment with human TFIIF subunits and indicate that the Tfg1 dimerization domain extends to residues 375-424.

Supplementary Figure 4.



Supplementary figure 4. Purification of Tfg1/Tfg2 dimer and transcription activity. (a) The purified recombinant TFIIF consisting of SmTfg1 and ScTfg2 subunits was analyzed by SDS-PAGE electrophoresis and visualized with Coomassie blue staining. Cysteine mutations of SmTfg1 are listed on top, and protein bands of SmTfg1 and ScTfg2 (Tfg2) are as indicated. Non-cys: TFIIF construct contains no cysteine. M: molecular weight marker. (b) In vitro transcription activity of the recombinant TFIIF. The purified non-cys TFIIF (rTFIIF) was used in the in vitro transcription assay with the TFIIF-depleted nuclear extract containing no detectable TFIIF based on Western analysis probing for Tfg2. The recombinant TFIIF provided similar level of transcription activity compared with the non-depleted nuclear extract containing wild-type TFIIF (data not shown). In vitro transcription assay was conducted as described in METHODS. The amount of recombinant TFIIF used in the transcription assay was 320 ng. (c) In vitro transcription activity of the FeBABE conjugated TFIIF. Same as in (b), the FeBABE conjugated TFIIF was used in the transcription assay with TFIIF-depleted nuclear extract. FeBABE (-/+): TFIIF cys mutants with or without FeBABE attachment.

Supplementary Figure 5.

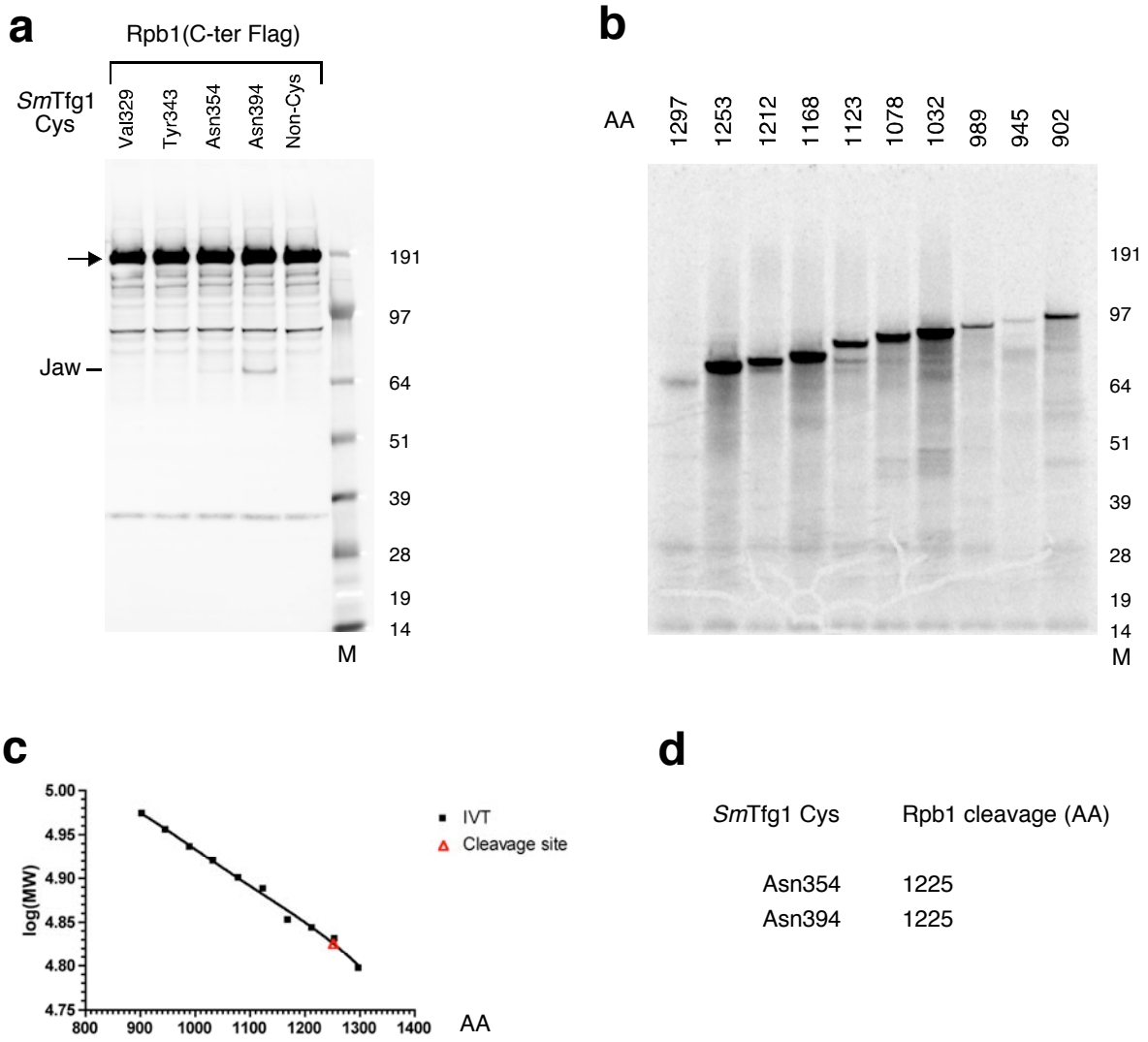


d

*Sm*Tfg1 Cys	Rpb2 cleavage (AA)
Val329	425, 371, 353, 331, 270
Tyr343	347, 212
Asn354	281
Ser359	347, 315
Asn364	556, 369, 152
Asn394	342, 308, 280

Supplementary figure 5. TFIIF-FeBABE hydroxyl radical protein cleavage and determination of the cleavage sites on Rpb2. (a) Western blot showing specific cleavage fragments of N-terminal Flag epitope tagged Rpb2 (Rpb2 N-ter Flag). Arrow indicates full-length Rpb2. Cysteine mutations in SmTfg1 are indicated. (b) In vitro translated peptide fragments of the N-terminal region of Rpb2 analyzed by SDS-PAGE and visualized by Phosphorimager. The C-terminal amino acid of Rpb2 is listed on top. (c) Calibration curve for determining the amino acid numbers of FeBABE cleavage fragments. The calibration curve is derived from a 4th order polynomial function using the sizes (log(MW)) of in vitro translated Rpb2 peptides (IVT) and their corresponding amino acid residues in (b). Locations of the FeBABE cleavage fragments on the calibration curves are shown with red triangles. (d) Table of calculated FeBABE cleavage sites in Rpb2 for TFIIF cys mutants.

Supplementary Figure 6.



Supplementary figure 6. TFIIF-FeBABE hydroxyl radical protein cleavage and determination of the cleavage sites on Rpb1. (a) Specific cleavage fragments of C-terminal Flag epitope tagged Rpb1 (Rpb1 C-ter Flag). Arrow indicates full-length Rpb1. Cysteine mutations in SmTfg1 are indicated. The cleavage fragment corresponds to a cleavage site in Rpb1 jaw domain (see below). (b) In vitro translated peptide fragments of the C-terminal region of Rpb1 analyzed by SDS-PAGE and visualized by Phosphorimager. The C-terminal amino acid of Rpb1 is listed on top. (c) Calibration curve for determining the amino acid numbers of FeBABE cleavage fragments. The calibration curve is derived from a 4th order polynomial function using the sizes (log(MW)) of in vitro translated Rpb1 peptides (IVT) and their corresponding amino acid numbers in (b). Locations of the FeBABE cleavage fragment on the calibration curve is shown with red triangles. (d) Table of calculated FeBABE cleavage sites in Rpb1 for TFIIF cys mutants.